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# Maturity Effects on Mineral Concentration and Uptake in Annual Ryegrass G. E. Brink<sup>a</sup>; K. R. Sistani<sup>b</sup>; J. L. Oldham<sup>c</sup>; G. A. Pederson<sup>d</sup>

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# Maturity Effects on Mineral Concentration and Uptake in Annual Ryegrass

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#### **ABSTRACT**

Annual ryegrass (*Lolium multiflorum* Lam.) provides livestock feed and captures nutrients from fields receiving manure application. The objective of this study was to determine relationships among maturity and yield, mineral uptake, and mineral concentration. Primary spring growth of 'Marshall' ryegrass was harvested every 7 d to 56 d maturity and was fertilized with swine effluent containing 254 and 161 kg nitrogen (N) and 42 and 26 kg phosphorus (P) ha<sup>-1</sup> for two years. Yield increased linearly to a maximum of 13.6 mg ha<sup>-1</sup> after 49 d in 2001 and 8.0 mg ha<sup>-1</sup> after 56 d in 2002. Mineral uptake was highly correlated (r > 0.95) with yield and attained a maximum single harvest of 192 kg N ha<sup>-1</sup> and 32 kg P ha<sup>-1</sup> (mean of two years). Concentration of all minerals except calcium (Ca) declined as ryegrass matured. Low magnesium (Mg) concentration (<2 g kg<sup>-1</sup> dry matter) increases the risk of hypomagnesemic grass tetany.

**Keywords:** ryegrass, livestock, mineral uptake, mineral concentration, nutrient management

#### INTRODUCTION

There has been significant growth in confined, contract swine production in many areas of the southeastern United States within the last decade (Welsh and Hubbell, 1999). Much of the manure effluent produced in the region is applied to bermudagrass [*Cynodon dactylon* (L.) Pers.] pastures and hayfields (Burton and Hanna, 1995). Because U.S. Natural Resource Conservation Service

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nutrient-management guidelines (code 590) stipulate that the timing of nutrient application should correspond as closely as possible with plant nutrient uptake (NRCS, 1999), effluent application is confined primarily to the summer months when bermudagrass is actively growing (Brink et al., 2003). Seeding a temperate forage species into tropical bermudagrass extends the period of active crop growth into the spring, providing a source of livestock feed (Bagley et al., 1988) and representing an opportunity to capture and export additional nutrients from fields receiving manure application (Evers, 2002; Rowe and Fairbrother, 2003).

Temperate forage species, which include annual ryegrass (*Lolium multi-florum* Lam.), annual and perennial clovers (*Trifolium* sP.), and small grains, are planted in late summer or early fall in the southeastern United States and are usually grazed during the winter and spring (Bagley et al., 1988). If a hay crop is desired, producers are usually limited to a single harvest in late spring because of frequent precipitation and low temperatures during March and April. Among temperate forages, annual ryegrass is the most widely utilized due to its adaptability to a broad range of soil and climatic conditions, ease of establishment, late maturity compared with small grains, and excellent forage quality (Balasko et al., 1995).

As with temperate perennial grasses (Collins and Casler, 1990), the nutritive value of annual ryegrass declines with increasing maturity. Valente et al. (2000) reported that the organic-matter digestibility of several annual ryegrass cultivars declined from approximately 900 to 650 g kg<sup>-1</sup> over 50 d of growth. This trend was also observed in the herbage phosphorus (P) and potassium (K) concentration of the annual grasses barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), and wheat (*Triticum aestivum* L.) (Cherney and Marten, 1982). Manure applied during maturation may influence mineral concentration, which has implications for livestock health. The objective of this study was to determine the relationships between maturity and yield, mineral concentration, and mineral uptake in annual ryegrass fertilized with swine effluent.

#### MATERIALS AND METHODS

The study was conducted on a confined-feeding swine farm in northeast Mississippi near Pheba (lat 33.6 N, long 88.9 W) on a Prentiss loam. At the farm, manure and urine are washed from pits beneath a slatted barn floor into open lagoons. Manure solids settle to the bottom and effluent is applied by a center-pivot irrigation system. Effluent had been applied at rates ranging from 10 to 15 cm ha<sup>-1</sup> yr<sup>-1</sup> (unknown mineral concentration) for three years before the experiments started. Soil mineral characteristics were determined using Mehlich-3 extractant (Mehlich, 1984) (Table 1). Total soil N concentration was determined by the Dumas method (Bremner, 1996).

The plot area was located within a 40–45 ha field; effluent was applied after the initial clipping and each of the first three harvests (0.6 cm ha<sup>-1</sup>

30 - 40

4.08

0.52

0.03

Mineral characteristics of the soil (Menlich-3) at the beginning of the experiment										
			$\rm g~kg^{-1}$			mg kg <sup>-1</sup>				
cm depth	pН	N	K	Ca	P	Cu	Fe	Mg	Mn	Zn
0–5	6.11	2.11	0.44	1.14	31	4	306	230	83	0.99
5-10	5.88	1.48	0.32	0.81	1	12	208	80	76	0.33
10-20	5.96	1.07	0.12	0.71	0	8	110	30	78	0.08
20-30	4.25	0.64	0.03	0.44	0	9	103	20	12	0.08

0.25

0 11

114

20

7

0.14

Table 1

Mineral characteristics of the soil (Mehlich-3) at the beginning of the experiment

application<sup>-1</sup>) using a portable tank and application equipment, and after the next four harvests at the producer's discretion in terms of frequency. Effluent application by the producer was greater in 2001 than 2002, which accounted for the difference in mineral application rate between the two years (Table 2). A 250 mL effluent sub-sample was collected at each application and was analyzed for total nitrogen (N) concentration by the Kjeldahl procedure with a salicylic-acid modification (Bremner, 1996). Total effluent P, K, calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), and zinc (Zn) concentration were determined by digesting duplicate 20 mL subsamples containing 1 mL concentrated H<sub>2</sub>SO<sub>4</sub> and 5 mL concentrated HNO<sub>3</sub> to a total volume of 1 mL, diluting with 40 mL distilled water, and filtering through Whatman 2V paper (Southern Cooperative Series, 1983). Mineral concentration of the filtrate was measured by emission spectroscopy on an inductively coupled, dual-axial argon plasma spectrophotometer. Total mineral quantity applied is presented in Table 2.

Marshall annual ryegrass was planted in rows (20 cm spacing) at 44.8 kg ha $^{-1}$  into 2  $\times$  4 m common bermudagrass plots in four replicates in early September of 2000 and 2001. On April 5, 2001 and April 10, 2002, plots were clipped to a 5 cm stubble and divided into eight 1  $\times$  1 m subplots, which were randomly assigned days of maturity at harvest. Beginning 7 d after the initial clipping, primary growth was harvested from a 0.75 m $^2$  section of each subplot

Table 2 Quantity of minerals applied each year with the swine effluent

	${ m mg~kg^{-1}}$									
 N	K	Ca	P	Cu	Fe	Mg	Mn	Zn		
					1.45 0.92					

every 7 d to 56 d maturity. Harvested ryegrass samples were dried in cloth bags at 60°C for 48 h, weighed to determine dry-matter yield, and ground to pass a 2 mm screen. Subsamples were ground to pass a 1 mm screen and stored in 150 mL plastic bottles.

Forage N concentration was determined by the Dumas method (Bremner, 1996). Forage P, K, Ca, Cu, Fe, Mg, Mn, and Zn concentrations were determined by ashing duplicate 0.8 gm sub-samples in a ceramic crucible at 500 EC for 4 h followed by dissoling the ash in 1.0 mL of 6 N HCl for 1 h and then in an additional 40 mL of a double acid solution of 0.025 N H<sub>2</sub>SO<sub>4</sub> and 0.05 N HCl for another hour, and then filtering it through Whatman no. 1 paper (Southern Cooperative Series, 1983). Mineral concentration of the filtrate was measured by emission spectroscopy on an inductively coupled, dual-axial argon plasma spectrophotometer.

Mineral uptake was calculated as the product of forage dry-matter yield and mineral concentration at each harvest. Data were subject to analysis of variance; regression analysis of the means from four field replicates was used to determine linear and nonlinear coefficients associated with changes in forage dry-matter yield, mineral concentration, and mineral uptake (Table 3).

Table 3
Regression equations relating yield, N and P uptake, and mineral concentration (mean of 2 yr) of ryegrass with days (D) of primary growth

Parameter		$\mathbb{R}^2$	RMSE
Yield			
2001	Y = -3.1 + 0.30D	0.93	1.6
2002	Y = -1.3 + 0.16D	0.97	0.5
N uptake			
2001	Y = -18.1 + 4.98D	0.90	30.9
2002	Y = -7.0 + 2.44D	0.90	14.8
P uptake			
2001	Y = -3.3 + 0.72D	0.91	4.3
2002	Y = -0.9 + 0.48D	0.91	2.8
Concentration			
N	$Y = 44.6 - 0.89D + 0.007D^2$	0.93	2.62
P	$Y = 7.9 - 0.18D + 0.002D^2$	0.99	0.17
K	Y = 46.7 - 0.38D	0.97	1.18
Ca	Y = 2.1 + 0.01D	0.99	0.02
Mg	Y = 1.7 - 0.01D	0.82	0.08
Mn	$Y = 0.078 - 0.0017D + 0.00002D^2$	0.92	0.003
Cu	$Y = 6.4 - 0.12D + 0.001D^2$	0.95	0.27
Fe	$Y = 114.7 - 3.44D + 0.052D^2$	0.75	8.78
Zn	$Y = 29.1 - 0.44D + 0.004D^2$	0.92	1.12
K/(Ca + Mg)	Y = 4.8 - 0.04D	0.98	0.09

Coefficients were included in a regression equation when the coefficient was significant ( $P \le 0.05$ ) and the increase in equation order increased the  $R^2$  by at least 5%. Pearson correlation coefficients between dry-matter yield and mineral uptake were calculated on an entry mean basis and are reported at  $P \le 0.001$ .

#### RESULTS AND DISCUSSION

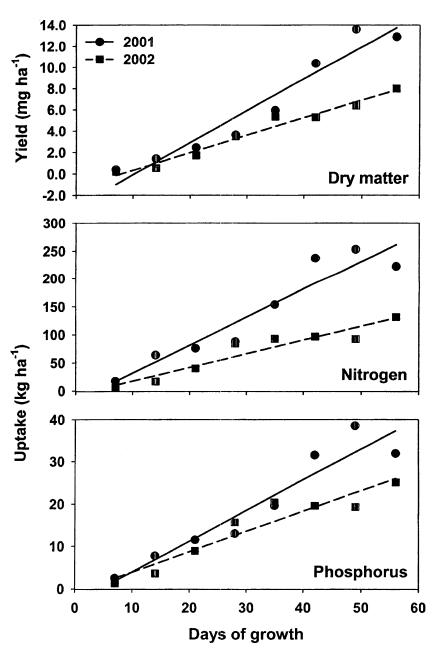
### **Dry-Matter Yield**

Ryegrass exhibited a linear trend for dry-matter yield in 2001 and 2002, reaching a maximum of 13.6 mg ha<sup>-1</sup> after 49 d of growth in 2001 and 8.0 mg ha<sup>-1</sup> after 56 d of growth in 2002 (Figure 1). Maximum yield and rate of dry-matter accumulation (0.30 versus 0.16 mg ha<sup>-1</sup> d<sup>-1</sup>; Table 3) were greater in 2001 than in 2002 due to greater effluent application rates by the producer, which resulted in greater N application rates (Table 2). Redfearn et al. (2002) found that late-maturing ryegrass cultivars such as 'Marshall' (Arnold et al., 1981) had a yield advantage over early maturing cultivars, due primarily to greater yield from April through the end of the growing season. Effluent application often begins during this period, and late-maturing cultivars would thus have the best potential to utilize the applied minerals.

The linear trends measured for dry-matter production in both years were similar to those measured in other temperate annual grasses receiving a single application of inorganic fertilizer (Cherney and Marten, 1982), and maximum yields were typical of those for this forage species (Redfearn et al., 2002). The increasing yield of successive harvests of ryegrass from early to late spring could be attributed to the increasing contribution of the stem and reproductive fractions to total herbage dry weight (Cherney and Marten, 1982; Redfearn et al., 2002).

## Nitrogen and Phosphorus Uptake

Similar to dry-matter yield, N and P uptake exhibited linear responses to increasing ryegrass maturity in 2001 and 2002 (Figure 1). Correlations between mineral uptake and dry-matter yield were significant in both years (r=0.96 in 2001 and 0.95 in 2002 for N; r=0.98 in 2001 and 0.97 in 2002 for P). Maximum single-harvest mineral uptake by ryegrass measured here (192 kg N ha<sup>-1</sup> and 32 kg P ha<sup>-1</sup>; mean of two years) exceeded that measured by Rowe and Fairbrother (2003) at the same location (106 kg N ha<sup>-1</sup> and 21 kg P ha<sup>-1</sup>, respectively, mean of three years), suggesting that N applied in effluent early in the growing season of this study (April 5, 2001 and April 10, 2002), before application was begun by the producer, had a positive impact on ryegrass mineral



*Figure 1.* Dry-matter yield and N and P uptake of ryegrass regressed on days of primary spring growth in 2001 and 2002. Refer to Table 3 for equations describing regression trends.

uptake. Evers (2002) found that N and P uptake by ryegrass following broilerlitter application was greatest when additional N was applied in December or March compared with May.

#### **Mineral Concentration**

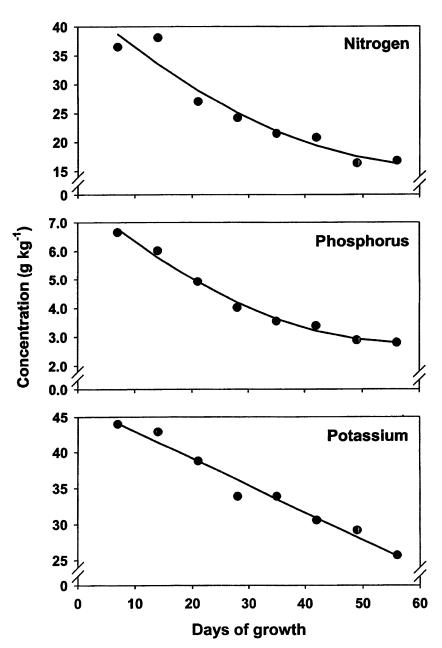
The effect of year on the concentration of any mineral was not significant as ryegrass matured during the springs of 2001 and 2002 (no significant year  $\times$  days of growth interaction). With the exception of Ca, concentration of all minerals declined as ryegrass matured from 7 to 56 d of growth. These trends generally agree with those reported by Casler et al. (1987) for smooth bromegrass (*Bromus inermis* Leyss.).

Nitrogen concentration of ryegrass herbage exhibited a nonlinear decline from 36.5 g kg<sup>-1</sup> at 7 d of growth to 16.8 g kg<sup>-1</sup> at 56 d of growth (Figure 2). Collins and Casler (1990) reported a similar trend for N concentration of smooth bromegrass (*Bromus inermis* Leyss), orchardgrass (*Dactylis glomerata* L.), timothy (*Phleum pratense* L.), tall fescue (*Festuca arundinacea* Schreb.), and reed canarygrass (*Phalaris arundinacea* L.) over 49 d of primary growth, as did Cherney and Marten (1982) for the small grains barley, oats, and wheat. In both of these studies, grasses were fertilized with 50 to 100 kg N ha<sup>-1</sup>, about one-half the N applied in this study in the effluent (Table 2), suggesting that annual ryegrass N concentration responds little to increasing N availability despite weekly N application.

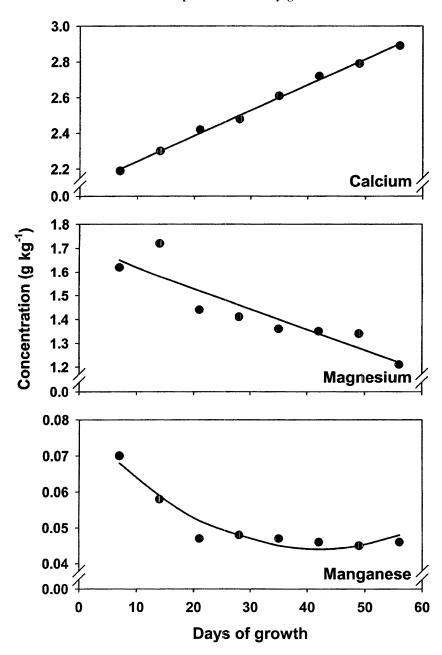
Ryegrass P concentration also exhibited a nonlinear trend during the spring, declining from 6.7 to 2.8 g kg $^{-1}$  (Figure 2). The P concentration of ryegrass when it would typically be harvested for hay (4.0–3.6 g kg $^{-1}$  at 28 to 35 d, respectively) was similar to that reported for a broad collection of temperate grasses (Minson, 1990). In contrast, the rate of decline in P concentration of ryegrass, or the linear component of the regression equation (-0.18 g kg $^{-1}$  d $^{-1}$ ; Table 3), was three-fold greater than that measured for smooth bromegrass (-0.06 g kg $^{-1}$  d $^{-1}$ ) by Casler et al. (1987).

Potassium concentration of ryegrass declined linearly from 44 g kg $^{-1}$  at 7 d to 26 g kg $^{-1}$  at 56 d of growth (Figure 2). Potassium concentration of swine effluent was considerably greater than that of the other minerals, which resulted in high application rates (Table 2). With the exception of the last sampling date, herbage K concentration was always greater than that necessary for 90% of maximum yield (28 g kg $^{-1}$ ) (Robinson, 1996).

Calcium concentration exhibited a linear increase during primary growth (Figure 3). The decline in mineral concentration typically observed with increasing maturity (Casler et al., 1987) was not evident here. Mean Ca concentration was, however, less than one-half of that reported for perennial ryegrass (2.6 vs. 5.9 g kg<sup>-1</sup>) (Minson, 1990). Burns et al. (1987) found that Ca concentration of tall fescue was reduced by fertilization with swine effluent compared with



*Figure 2.* Ryegrass N, P, and K concentration regressed on days of primary growth (mean of 2 yr). Refer to Table 3 for equations describing regression trends.



*Figure 3.* Ryegrass Ca, Mg, and Mn concentration regressed on days of primary growth (mean of 2 yr). Refer to Table 3 for equations describing regression trends.

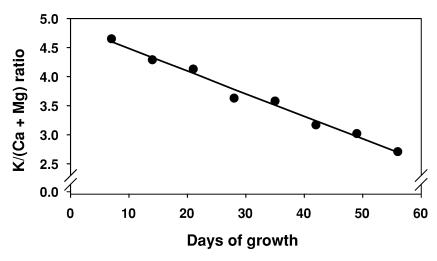
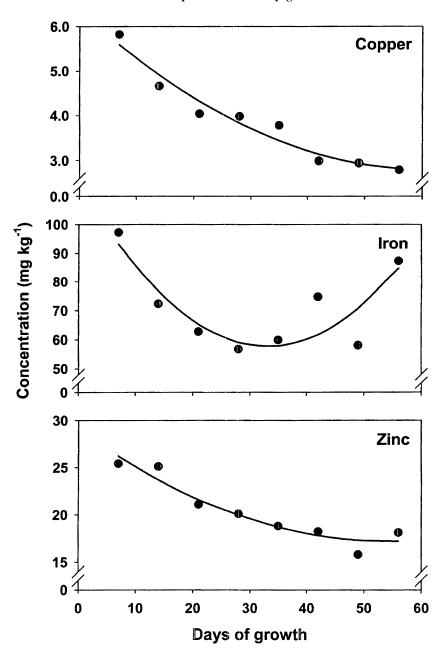


Figure 4. Ratio of ryegrass K to Ca + Mg concentration regressed on days of primary growth (mean of 2 yr). Refer to Table 3 for equations describing regression trends.

inorganic fertilizer. In addition, Ca concentration of temperate grasses tends to be lower in the spring than in the summer and fall (Minson, 1990).

Magnesium concentration of ryegrass exhibited a linear decline during primary spring growth (Figure 3) and was always lower than the 2 g kg<sup>-1</sup> dry matter considered adequate for general diets of ruminants. Magnesium deficiency in forages is frequently associated with immature forage and with excessive K fertilization, factors present here, which could produce hypomagnesemic grass tetany in ruminants. This condition is characterized by low blood-plasma Mg concentration (<0.4 mmol L<sup>-1</sup>) and is generally found in cows and ewes near parturition (Mayland and Wilkinson, 1996). The risk of grass tetany in ruminants increases as herbage K/(Ca+Mg) increases above 2.3. In this study, K/(Ca+Mg) declined linearly from 4.6 at 7 d to 2.7 at 56 d of growth (Figure 4), indicating that ryegrass fertilized with swine effluent in the manner used here has a high risk of producing grass tetany, particularly when utilized at early stages of spring growth.

The trace minerals Mn, Cu, Fe, and Zn are found in relatively small concentrations in forages, but are essential elements in the diet of ruminant livestock. Manganese concentration generally remains relatively constant as forage matures. In this study, Mn concentration of ryegrass exhibited a quadratric decline with maturity (Figure 3), but was relatively unchanged after 21 d, ranging from 0.045 to 0.048 g kg<sup>-1</sup>, or approximately twice the concentration considered necessary for optimal growth and reproduction (0.020–0.025 g kg<sup>-1</sup>) (Minson, 1990). Copper concentration declined with maturity (Figure 5), but always exceeded the minimum necessary for ruminant livestock (3 mg kg<sup>-1</sup>). In contrast,



*Figure 5.* Ryegrass Cu, Fe, and Zn concentration regressed on days of primary growth (mean of 2 yr). Refer to Table 3 for equations describing regression trends.

Fe concentration declined from 97 to 57 mg kg<sup>-1</sup> at 28 d of maturity, but then increased to 87 mg kg<sup>-1</sup> at 56 d of maturity (Figure 5), probably as a result of the increasing contribution of the reproductive (seed) fraction. Small grains and some temperate grasses harvested near maturity for silage or hay have high Fe concentration compared with those at less mature stages (NRC, 1996). Similar to Cu, Zn concentration declined during primary growth, but as others have reported (Minson, 1990), only over a relatively small range (25 to 18 mg kg<sup>-1</sup>) (Figure 5).

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